

Problem 1) A researcher was interested in a quinone which she thought might be involved in photosynthetic production of ATP. Such quinones undergo a redox reaction  $Q + 2H^+ + 2e^- \rightarrow QH_2$ . She wanted to determine the energetics of this reaction. She had only the reduced quinone  $QH_2$  and she decided to use an electrochemical cell. On one side she put the  $QH_2$  at 1 mM concentration in a buffered pH=5 water solution with a Pt electrode, and on the other side she put a copper electrode into 1 M  $Cu^{+2}$  solution. She had looked up the copper half cell reduction  $Cu^{+2} + 2e^- \rightarrow Cu$   $E^0 = +0.337$  V. She connected the half cells with a wire, and let it stand until equilibrium was reached. Spectroscopically she could determine that the concentration of  $QH_2$  was then 0.1  $\mu$ M.

a) What is the overall balanced reaction which was occurring while the cell was running?

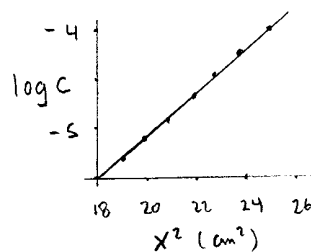
b) What is the  $\Delta G^0$  value for the conversion of Q to  $QH_2$ ? (state any assumptions you need)

c) The  $\Delta G$  value for ATP hydrolysis ( $ATP + H_2O \rightarrow ADP + PO_4$ ) is about -41 kJ/mol under normal conditions in the cell. What concentration ratio of  $QH_2$  to Q would be required under cellular conditions (pH=7) to drive synthesis of one mole of ATP?

Problem 2) A graduate student was studying a bacteriophage protein called the Cro repressor, which binds DNA to regulate the expression of a set of genes. He was unsure about whether the protein would bind the DNA as a monomer, dimer or tetramer. He knew that there were 65 amino acid residues in the protein (which would mean a monomer molecular weight of about 7,000 g/mol or 7 kD). He made up a solution of 2 mg of the protein in 1 ml of buffer and determined that the osmotic pressure (with a membrane that blocked only passage of protein) was 0.0035 atm at 25°C. He also did equilibrium centrifugation, and found the concentration profile shown at the right below.

a) Based on the osmotic pressure data what is the apparent molecular weight of the protein?

b) Based on the centrifugation data what is the apparent molecular weight of the protein?  
(for this centrifuge run  $T=25^{\circ}\text{C}$ ,  $\omega=24,500$  rpm,  $\bar{v}_2=0.710$  cm<sup>3</sup>g<sup>-1</sup>,  $\rho=1.05$  g cm<sup>-3</sup>)



c) What do you conclude about the dominant form of the protein in solution?

Problem 3) To understand more about the interactions of peptides with membranes Prof. Shin's group has used ESR (electron spin resonance) spectroscopy to look at partitioning of a set of peptides between water and a membrane which has a hydrophobic interior. The peptides vary in sequence at just one amino acid, X. (the peptides are M L S C R Q X I R F F K P A T R T L S S S S R Y L L) With this technique it is possible to measure the amount of peptide free in solution, and the amount bound to membranes. Results are given for three peptides below, all with total peptide concentration of  $77\mu\text{M}$ , at 294 K, and a constant amount of membrane.

	<u>bound</u>	<u>free</u>
X = Gly	22 $\mu\text{M}$	55 $\mu\text{M}$
X = Ala	27 $\mu\text{M}$	50 $\mu\text{M}$
X = Leu	40 $\mu\text{M}$	36 $\mu\text{M}$

a) Using these data what is standard free energy for binding of the Leu peptide to membrane?

b) Using these data, estimate the free energy of **transfer** of the **sidechain** of the amino acid Leu from water to the membrane.

c) Other ESR data show that the sidechains of these amino acids are being buried in the region of the membrane made up of the long alkyl chains of the fatty acids. Do you think there would be a correlation between the amino acids which best bind to membranes and those which are best (i.e. lowest free energy) in the interior of a protein? (explain)